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Chondrogenic pharmacodynamics of rhGDF-5 dose and exposure duration

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Purpose: Growth and differentiation factor-5 (GDF-5) is a TGF-b superfamily signaling molecule that regulates chondrogenesis and limb development. In the current study, rhGDF-5 pharmacodynamic activity was evaluated using an in vitro chondrogenic assay to explore time-dependent enhancement of cartilage matrix synthesis and cell proliferation. Various growth factor doses and exposure durations were evaluated to establish a model system for predicting in vivo pharmacodynamics.
Methods and Materials: Primary chondrocyte pellets were cultured in a serum-free, chemically-defined media supplemented with rhGDF-5 (1.4-1000 ng/ml) for 3, 7, 14, or 21d. Some cultures received transient rhGDF-5 exposure for only 3, 7, or 14d. Media was changed twice weekly. Matrix accumulation and cell proliferation were quantified to identify peak response, minimal effect dose (MED), and EC~50~.
Results: Continuous rhGDF-5 supplementation demonstrated dose and time-dependent increases in sGAG content. Peak levels were achieved at 111 ng/ml, the MED decreased with culture duration (from 111 ng/ml (3d) to 4 ng/ml (21d), $p<0.001$), and the EC~50~ was 24 ng/ml at 21d. Increased cell proliferation (~2-fold) occurred at all time points but only at doses >37 ng/ml ($p<0.001$). Transient rhGDF-5 exposure for 14d provided equivalent dose-response characteristics at 21d as cultures treated continuously. Exposure for only 7d provided comparable peak sGAG accumulation at 1000 ng/ml (21d); however, the MED (37 ng/ml) and EC~50~ (210 ng/ml) were significantly higher ($p<0.001$) than those observed with continuous supplementation.
Conclusions: These results provide new insight into rhGDF-5 chondrogenic pharmacodynamics and establish a model for predicting in vivo efficacy of combinatorial therapeutic strategies targeting growth factor dose and exposure duration.

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Transplanted allogenic chondrocytes simultaneously overexpressing human insulin-like growth factor I (IGF-I) and fibroblast growth factor-2 (FGF-2) stimulate articular cartilage repair in vivo

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Purpose: Transplantation of genetically modified articular chondrocytes overexpressing either human IGF-I or FGF-2 enhances the repair of osteochondral defects in vivo. We tested the effect of articular chondrocytes transfected with a combination of IGF-I and FGF-2 on the repair of osteochondral defects in vivo.
Methods and Materials: Chondrocytes were transfected with the E. coli lacZ gene or a combination of a human IGF-I and FGF-2 cDNA (IGF-I/FGF-2) using FuGENE 6, encapsulated in alginate and cultured for 21 days in vitro. IGF-I and FGF-2 concentrations were measured by ELISA. Alginate-chondrocyte spheres were press-fit into osteochondral defects (n=2) in each patellar groove of 14 rabbits. Three weeks post operation, cartilage repair was histologically graded based on 1&0 safranin-O-stained sections. Proteoglycan content of the repair tissue was measured using the DMMB assay. A mixed model analysis of variance was used to compare groups to account for multiple slides nested within the same animal.
Results: IGF-I and FGF-2 expression levels were significantly higher than in lacZ spheres until at least day 7. Combined gene transfer significantly improved filling of the defect, integration, matrix staining, cellular morphology, architecture of the defect and the surface and new subchondral bone formation. Average total score after 3 weeks in vivo was significantly improved for IGF-I/FGF-2 defects (15.97) compared with lacZ defects (21.91) ($P < 0.001$). Proteoglycan content of the repair tissue was 2.1-fold increased in IGF-I/FGF-2 defects ($P = 0.02$).
Conclusions: The data demonstrate that the combined overexpression of IGF-I and FGF-2 within osteochondral defects significantly enhances the repair after 3 weeks in vivo.

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Effect of TGF-β1 and IGF-1 in an "in vitro" model of avascular meniscal lesions treated with different support materials

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Purpose: In vitro repair analysis of cylindrical lesions in the avascular zone of the meniscus by treating them with growth factors TGF-beta and IGF-1, and filled with different support materials
Methods and Materials: Avascular cylindric holes performed in the avascular zone of the meniscus were filled with cylinders of fresh meniscus, frozen meniscal allografts, and artificial collagen sponges cultivated with and without cartilage cells. All the groups were treated with TGF-beta1 and IGF-1 and analyzed after 2, 4, 6 and 8 weeks
Results: TGF-beta1 and IGF-1 induced the appearance of tissue unions between the filling structure and the edges of the lesion, as well as an increase in cell proliferation in meniscal cells. We also observed the presence of cells from the meniscus inside to the collagen sponges used, which was potentiated by the presence of TGF-beta1 and IGF-1. Cell presence in the sponge does not bear any correspondence with processes of proliferation.
Conclusions: Filling the meniscal lesions with meniscal tissue or collagen matrices combined with TGF-beta1 and IGF-1 was useful to improve cell response processes in the experimental model used

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A regenerative approach for osteochondral defects using delivered recombinant cartilage derived retinoic acid sensitive protein (CD-RAP)

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Purpose: Effective regeneration for traumatic cartilage damage options are not jet available. CD-RAP is a highly specific marker for cartilage and controls chondrogenic differentiation and maintenance. It was evaluated that CD-RAP improves cartilage healing in an animal model for critical size osteochondral defects.
Methods and Materials: In 28 skeletally mature New Zealand White rabbits a critical size defect of 3 mm diameter and 3.5 mm depth were prepared in the middle of the trochlea. During the formation of the autologous blood clot 4 µg rhCD-RAP delivered within a collagen sponge were implanted. After an in life phase of 8 weeks the analysis was performed macroscopically and histologically by the Morris score.
Results: The treatment with 4 µg rhCD-RAP immobilized on collagen statistically significantly enhance the formation of new cartilage compared to sham and vehicle control in the histological evaluation by the Morris score. The average scores for sham, vehicle control and treatments group with CD-RAP immobilized on collagen were 16.5 ± 2.9, 16.2 ± 6.1, 8.1 ± 3.7. CD-RAP delivered on collagen showed a significant improvement over the vehicle and sham group.
Conclusions: Results indicate that CD-RAP delivered on collagen in the defect site offers a new therapeutic approach for the regeneration of osteochondral defects.